Making the Cut: Options for Making Initial Evaluations of Malting Quality in Barley[1]

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ABSTRACT

Alternatives to traditional methods of estimating the potential malting quality of barley lines were examined through their application to two regional barley nurseries, with the goal of identifying streamlined procedures suitable for making initial assessments of malting quality. Mashing malt using an isothermal hot water extract (HWE) procedure instead of the standard Congress mash generated distinct but closely related values for primary measures of malt modification, including malt extract, wort soluble protein, soluble/total malt protein, wort β-glucan, and wort free amino nitrogen. Simple measurements of other wort properties, including refractive index and wort osmolyte concentration, also showed strong correlations between Congress and HWE worts. Analyses of small-scale (microcentrifuge tube) versions of isothermal HWE worts showed good correlations with the attributes of Congress worts. Although the key malting quality metrics for Congress and HWE worts were not equivalent, their close correlations suggest that they may be equally able to differentiate the malting quality of barley lines. The simple isothermal temperature profile of the HWE wort, in conjunction with implementation of methods that use simple laboratory instrumentation for wort analysis, could simplify initial malting quality analysis and make it more accessible to laboratories lacking dedicated mashing and wort analysis instruments.

Keywords: Barley breeding, Malting barley, Malting quality analysis

RESUMEN

Las alternativas a los métodos tradicionales de estimación de la calidad potencial de las líneas de cerveza de cebada fueron examinados a través de su aplicación a dos viveros de cebada regional con el objetivo de identificar los procedimientos adecuados simplificado para la determinación inicial de calidad maltera. Maceración de la malta con un extracto de agua caliente isotérmica (HWE) procedimiento en lugar de la norma puré Congreso generados, distintos pero estrechamente relacionados con los valores de las medidas primarias de la modificación de malta, incluyendo el extracto de malta, proteína soluble en mosto, soluble/total proteína de la malta, β-glucano, y amino nitrógeno libre del mosto. Mediciones simples de las propiedades del mosto, incluyendo el índice de refracción y la concentración osmolito del mosto, también mostraron una fuerte relación entre el Congreso y mostos HWE. Los análisis de pequeña escala (de tubo de microcentrífuga) versiones de mostos isotérmica HWE mostró una buena correlación con los atributos de mostos Congreso. A pesar de las métricas clave de calidad maltera del Congreso y de mostos HWE no eran equivalentes, sus correlaciones cerca sugieren que pueden ser capaces de diferenciar igualmente la calidad de las líneas de malta de cebada. El perfil de temperatura isotérmica simple del mosto HWE, en relación con los métodos implementados que uso en laboratorio de instrumentación sencilla para el análisis del mosto, puede simplificar el análisis inicial de calidad maltera y hacerlo más accesible a los laboratorios que falta instrumentación dedicados para la análisis de maceración y de mosto.

Palabras claves: Análisis de calidad maltera, Cebada malteada, Crianza de la cebada

Malted barley is one of the key materials used in brewing beer because it provides simple sugars and amino acids that support fermentation by yeast, along with flavor, color, and other sensory components. Barley grain is malted through a process of controlled germination and early seedling growth. Malt is produced by a three-step process that starts with hydrating the grain to a uniform moisture level (steeping). The hydrated grain is then incubated under controlled temperature, humidity, and airflow conditions for several days (typically about 4 days) with intermittent mixing of the grain bed. During this germination period (more accurately a period of early seedling growth), the grain produces a number of enzymes that can hydrolyze carbohydrates, proteins, and cell walls. These enzymes act on the various grain components, producing simple sugars, amino acids, and other compounds. During the malting process, the physical state of the kernel is also altered (modified), making it more friable or easily milled. At the desired state of modification, barley seedlings are heated (kilned) to stop seedling growth processes, dry and stabilize the malt, and preserve desired enzymatic, flavor, and other properties.

Although there is no single metric for malting quality due to differences in beer styles and brewing processes, the malting and brewing industries have developed a series of standard tests that measure the characteristics of barley, malt, and worts. Both the American Society of Brewing Chemists (ASBC) and the European Brewery Convention (EBC) have published compendia of methods (1,9) that contain nearly 50 (ASBC) and more than 70 (EBC) individual protocols for quantifying different aspects of barley, malt, and wort composition or properties. As Briggs (4) notes, various methods of malt analysis provide the brewer with a means to specify a consistent raw material and establish a basis of malt valuation, while also providing the maltster a source of data for process adjustment and quality control. Due to their importance in commerce, official ASBC and EBC methods of analysis are carefully evaluated for consistency both within and between laboratories to ensure uniform application and results and, hence, comparability within the industry. The various methods are not designed necessarily to reflect any individual brewer's or maltster's processes, but rather to provide a benchmark assessment that brewers and maltsters can interpret to predict the performance of a malt in their particular processes.

Aspects of malting quality analysis also play a major role early in the supply chain, when growers decide what barley varieties to plant and maltsters decide which crop varieties to purchase. Because barley varieties differ significantly in the characteristics of the malt that they will yield (2) and because many barley varieties are unsuitable for malt production, only grain from varieties known to malt well will be purchased for malt production.

Development of new malting barley varieties involves the evaluation of a number of attributes in addition to malting quality. New varieties must be at least competitive, if not superior, in their ag-

[1] Mention of trade names or commercial products in this presentation is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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ronomic performance and disease resistance characteristics, in addition to maintaining or improving malting performance, for producers to be willing to grow a new variety. Some agronomic characteristics (e.g., stalk height and strength, heading date, lodging resistance) can be evaluated in early generations during the selection process. Lines with clear deficiencies in these characteristics that are identified in early generations can be eliminated from further development efforts, allowing breeders to devote their resources to lines with better development potential. Lines selected for further development are grown in larger plots, often in multiple locations, allowing yield estimates and malting quality evaluations of the harvested grain. Initial malting quality analyses usually require greater quantities of grain (>50 g) than are generally available in the early generations of a selection program.

Prospective malting quality lines are tested for a standard set of malt quality parameters for several years during development, culminating, if successful, with inclusion in comparative regional nurseries. Routine initial analyses of malting quality in line development include a number of physical attributes: measurement of kernel weight, kernel size, barley color, and protein content of grain and malt; characterization of standard worts for characteristics such as malt extract, wort soluble protein, wort β-glucan, and wort color and clarity; and enzyme activity estimates, including diastatic power and α-amylase activity. Additional wort analyses (fine/coarse extract difference, viscosity, free amino nitrogen, turbidity, and pH) are conducted on samples from regional nurseries. Such regional nurseries include malting quality check varieties grown in a common environment with the new lines, enabling direct comparison of malting quality performance. Standard analyses follow a number of ASBC methods, including Barley-2D, -7C, and -9; Malt-2B, -4, -5A, -6C, -7B, and -8B; and Wort-8, -9, -12, -13, -17, -18, as appropriate (1). Due to the complexity of analyses and the specialized instrumentation and skills required, malting quality analyses are generally conducted in dedicated malting QA laboratories.

As currently implemented, the evaluation of malting quality in the United States consistently analyzes barley lines from their earliest QA assessment through the final stages of line development to the point where they are included in regional nurseries. By the time a prospective malting barley line is considered for inclusion in pilot- or plant-scale evaluations by the malting and brewing industries to determine acceptance as a recommended malting variety, it will have been tested over a number of years from seed lots produced in multiple locations, providing a broad and valuable perspective on the line's malting performance and consistency of results. At the same time, though, the detailed characterization of all lines submitted for malting quality analysis also results in substantial resources being expended in providing detailed malting quality profiles of many lines that will never be advanced to regional nurseries nor submitted for evaluation by the malting and brewing industries. While such detailed malting quality characterizations are essential for decisions about advancing a line into commercial evaluation, they may be less necessary for the simpler, initial decision of whether a line shows sufficient malting quality potential for its retention and advancement in a breeding program. Simplifying analyses to answer this more basic question could allow better utilization of available malting QA resources, while still providing useful data for early-stage decisions by breeders. Such simplifications could also enhance access of laboratories to malting quality analysis.

Historically, breeding line evaluations for malting quality have used the Congress mash (CM) as the standard method for generating wort for analysis. The protocol calls for grist to be suspended in water and held at 45°C for 30 min, followed by ramping the temperature to 70°C over 25 min (1 degree Celsius/min), holding at 70°C for 1 hr, and then cooling to room temperature within 10–15 min, requiring just over 2 hr for the process. This profile is not meant to reflect any individual brewer's commercial brewing protocols, but rather includes a number of features to accommodate processes considered important in mashing, including lower temperatures for initial proteolytic action, a gentle temperature ramp that allows some thermolabile carbohydrate-degrading enzyme activity, as well as a period at an elevated temperature for starch gelatinization and final conversion. Although there have been calls to abandon CM for other mashing protocols that better reflect brewery conditions (22), the CM protocol is still the de facto standard for malt specifications and breeding targets.

A less commonly used protocol, hot water extract (HWE), requires amounts of grist and water similar to CM but uses a simpler isothermal protocol, with the grist and water held at 65°C for 60 min followed by cooling to room temperature. As a result, HWE requires approximately half the time of CM. Although the two mashing protocols result in different absolute measures of malt extract or other wort components depending on the temperature sensitivities of the malt enzymes, it is not clear whether the two mashing protocols differ significantly in their utility for evaluating the relative malting potential of a given barley line. Therefore, the relative ability of the two mashing protocols to identify relatively better or poorer malts is unclear.

Another important aspect of malting quality analysis, beyond wort generation and characterization, is analysis of carbohydrate mobilization processes. Recently, several research groups have reexamined these aspects of malting quality assays and have proposed new measurements that are thought to improve the information provided to assess malting potential. Evans et al (10–13) suggest that replacing the single measurement of diastatic power with assays for the individual enzymes associated with starch hydrolysis provides a better estimate of a variety's starch conversion capabilities. In parallel, Duke and Henson, in a series of reports (5–7,15–17), show that measuring the osmotic potential of barley, malt, and malt extracts (essentially reflecting solute [largely sugar] concentrations) correlates well with carbohydrate enzyme activities, sugars produced, and other measures of the physiological state of the grain. In essence, this approach emphasizes measurement of the quantity of end products of carbohydrate mobilization rather than enzymatic capacity. This approach has the additional advantage of extreme simplicity of measurement, requiring only a single instrumental reading of a few microliters of wort or tissue extract. Taken together, the Evans and Duke and Henson groups are examining whether optimization and extension of traditional measurements or introduction of novel measurements of carbohydrate mobilization potential can provide more meaningful information on the malting quality potential of barley germplasm than is provided by current standard malt quality measurements.

In this paper, we reexamine mashing and analytical procedures, not to find a better predictor of brewhouse performance, but rather to determine whether simplified malting quality analyses can provide information that is predictive for differentiating the malting quality potential of barley lines.

EXPERIMENTAL

Germplasm

The barley lines used in this study were selected from the Mississippi Valley (MVN) and Western Regional Spring Barley (WRSBN) nurseries from the 2008 crop year. Lines included both 2- and 6-row named varieties (malting and feed type) and experimental lines and were grown in multiple locations. Line identification, malting quality data and summaries, and related information for the nurseries can be found online on the CCRU website at www.ars.usda.gov/SP2UserFiles/Place/36551000/barleyreports/2008MVN.pdf and www.ars.usda.gov/SP2UserFiles/Place/36551000/barleyreports/2008WRSBNQA.pdf

Malting

Lines were processed in micromalters (Joe White) according to protocols described previously (www.ars.usda.gov/SP2UserFiles/Place/36551000/barleyreports/2008MVN.pdf; 3) for 170 g (db) of grain. Samples were hydrated to 47% moisture using a 31-hr steep at 19°C: 8 hr wet, 8 hr air, 4 hr wet, 5 hr air, 2 hr wet, 2 hr air, and 2 hr wet. Larger barley grains (>42 mg/kernel) received a continuous wet presteep of between 2 and 7 hr at 16°C. The samples were germinated for 48 hr at 18°C, 24 hr at 17°C, and 24 hr at 16°C, with manual moisture adjustments to 47% at 0, 24, and 48 hr. Samples received two full turns every 1 hr. The germinated grain was kilned for 24 hr as follows: 10 hr at 49°C, 4 hr at 54°C, 3 hr at 60°C, 2 hr at 68°C, and 3 hr at 85°C, with 30-min ramps between stages. All stages received 40% total flow, with 0% recirculation for stages 1–3, 50% recirculation for stage 4, and 75% recirculation for stage 5.

Sample Grinding

Kilned, cleaned malt was ground using one of three methods. CM and traditional-scale HWE were prepared from a standard fine-grind malt grist using a cone-type mill (Miag-Seck) as specified in ASBC Malt-4 (1). In some experiments, malts were ground to the same ASBC Malt-4 (1) specifications (10 ± 1% retention on a 3-in. diameter, no. 30 screen) on a burr-mill style household coffee grinder (Capresso 560) adjusted to the second finest setting. For small-scale HWE, malt was ground using a small-scale ball mill (Mini BeadBeater-96, BioSpec) (19) with two 6.25-mm stainless-steel balls and 0.2 g of malt in a 2.0-mL microcentrifuge tube. The malt was homogenized to a fine powder with minimal husk fragments by processing for two cycles of 30 sec, with cooling on ice between processing cycles.

Sample Extraction

CM and HWE were prepared using a low gravity ratio (1:8) of grist to water. Standard-scale CM followed established ASBC Malt-4 protocols (1), with 25 g (db) of malt and 200 mL of added water. Standard-scale HWE (Institute of Brewing [IoB] method 2.3 [18]) used a comparable 25 g (db) of malt and 200 mL of water. Small-scale HWE was conducted in 2.0-mL microfuge tubes containing 187.5 mg of malt and 1.5 mL of water, analogous to the small-scale CM procedure described previously (21). Tubes containing grist and water at room temperature were placed in a preheated (67°C) thermomixer (Eppendorf Thermomixer-R) and incubated with continuous mixing for 60 min at 65°C after the block tem-

TABLE I
Malting Quality Score Criteria for 2- and 6-Row Barley Using Congress Mash and Hot Water Extract

	Congress Mash				Hot Water Extract			
	2-Row Barley		6-Row Barley		2-Row Barley		6-Row Barley	
Parameter	Range	Score	Range	Score	Range	Score	Range	Score
Kernel weight (mg)	>42.0	5	>32.0	5	>42.0	5	>32.0	5
	40.0–42.0	4	30.0–32.0	4	40.0–42.0	4	30.0–32.0	4
	38.0–40.0	2	28.0–30.0	2	>38.0–<40.0	2	>28.0–<30.0	2
	≤38.0	0	≤28.0	0	≤38.0	0	≤28.0	0
Assortment on 6/64 in. (%)	≥90.0	5	≥80.0	5	≥90.0	5	≥80.0	5
	85.0–89.9	3	73.0–79.9	3	85.0–<90.0	3	73.0–<80.0	3
	<85.0	0	<73.0	0	<85.0	0	<73.0	0
Malt extract (%)	≥81.0	10	≥79.0	10	≥79.2	10	≥77.0	10
	79.4–81.0	7	78.2–78.9	7	78.0–<79.2	7	76.2–<77.0	7
	78.0–79.4	4	77.7–78.2	4	76.0–<78.0	4	75.5–<76.2	4
	<78.0	0	<77.7	0	<76.0	0	<75.5	0
Wort clarity[a]	3	0	3	0	3	0	3	0
	2	1	2	1	2	1	2	1
	1	2	1	2	1	2	1	2
Barley protein (% db)	≥13.5	0	≥14.0	0	≥13.5	0	≥14.0	0
	13.0–13.5	5	13.5–13.9	5	13.0–13.5	5	13.5–13.9	5
	11.0–13.0	10	11.5–13.5	10	11.0–13.0	10	11.5–13.5	10
	≤11.0	5	≤11.5	5	≤11.0	5	≤11.5	5
Wort protein (% db)	>6.0	0	>6.0	0	>5.6	0	>5.6	0
	5.6–6.0	3	5.7–6.0	3	5.2–5.6	3	5.2–5.6	3
	4.4–5.6	7	5.2–5.7	7	4.2–<5.2	7	4.7–<5.2	7
	4.0–4.4	3	4.8–5.2	3	3.8–<4.2	3	4.4–<4.7	3
	<4.0	0	<4.8	0	<3.8	0	<4.4	0
S/T (soluble/total) protein	>47	0	>47	0	>44	0	>44	0
	40–47	5	42–47	5	37–44	5	39–44	5
	<40	0	<42	0	<37	0	<44	0
Diastatic power (°ASBC)	>120	7	>140	7	>120	7	>140	7
	100–120	4	120–140	4	100–120	4	120–140	4
	<100	0	<120	0	<100	0	<120	0
α-Amylase (20° DU)	>50	7	>50	7	>50	7	>50	7
	40–50	4	40–50	4	40–50	4	40–50	4
	<40	0	<40	0	<40	0	<40	0
β-Glucan (ppm)	<100	7	<120	7	<130	7	<165	7
	100–150	3	120–170	3	130–180	3	165–215	3
	>150	0	>170	0	>180	0	>215	0

[a] Wort clarity score: 1 = clear; 2 = slightly hazy; and 3 = hazy.

perature recovered to 65°C (approx. 2 min after tubes were placed in the preheated block). After 60 min of incubation, the tubes were removed and cooled to room temperature (approx. 22°C). After small-scale HWE, the tubes were centrifuged for 5 min at 15,000 × *g* to sediment the solids. The supernatant was collected by careful aspiration using a fine-tip disposable pipette. Reduced-scale samples were mashed in triplicate, with each replicate analyzed individually. Standard-scale CM and HWE mashing and analyses were performed on single, unreplicated samples, which is the standard practice in our QA program.

TABLE II
Comparison of Correlations of Wort Parameters for 25-g Hot Water Extract (HWE) and 25-g Congress Mash for 108 Entries from the Mississippi Valley Regional Barley Nursery from Crop Year 2008[a]

	Congress Mash[b]											
Fine-Grind Mill HWE[b]	**Malt Extract**	**RI**	**OC**	**Wort Protein**	**S/T Protein**	**β-Glucan**	**FAN**	**Color**	**Clarity**	**Mean**	**Min**	**Max**
Malt extract	**0.975**	0.862	0.555	0.292	0.684	–0.218	0.376	–0.126	–0.173	78.1	71.6	82.3
	***	***	***	$P = 0.002$	***	$P = 0.023$	***	$P = 0.196$	$P = 0.075$			
RI	0.944	**0.884**	0.648	0.431	0.773	–0.337	0.485	–0.102	–0.245	1.34598	1.34482	1.34666
	***	***	***	***	***	$P = 0.0004$	***	$P = 0.296$	$P = 0.011$			
OC	0.644	0.722	**0.843**	0.789	0.863	–0.510	0.770	–0.135	–0.503	262	210	291
	***	***	***	***	***	***	***	$P = 0.166$	***			
Wort protein	0.260	0.438	0.783	**0.988**	0.690	–0.436	0.918	–0.074	–0.571	4.92	2.86	6.54
	$P = 0.007$	***	***	***	***	***	***	$P = 0.449$	***			
S/T protein	0.661	0.744	0.785	0.748	**0.986**	–0.378	0.759	–0.022	–0.443	40.2	23.9	50.5
	***	***	***	***	***	***	***	$P = 0.822$	***			
β-Glucan	–0.166	–0.149	–0.296	–0.386	–0.327	**0.975**	–0.315	0.234	0.373	209	46	1,350
	$P = 0.086$	$P = 0.124$	$P = 0.002$	***	$P = 0.001$	***	$P = 0.001$	$P = 0.015$	***			
FAN	0.331	0.497	0.734	0.926	0.778	–0.421	**0.891**	–0.041	–0.473	170	81	258
	$P = 0.001$	***	***	***	***	***	***	$P = 0.675$	***			
Color	–0.140	0.010	–0.104	–0.006	0.144	0.200	–0.039	**0.557**	0.315	2.2	1.2	6.6
	$P = 0.150$	$P = 0.919$	$P = 0.286$	$P = 0.951$	$P = 0.139$	$P = 0.039$	$P = 0.690$	***	$P = 0.001$			
Clarity	–0.241	–0.299	–0.465	–0.438	–0.337	0.436	–0.430	0.556	**0.638**	1.31	1	3
	$P = 0.012$	$P = 0.002$	***	***	$P = 0.0004$	***	***	***	***			
Mean	80.1	1.34633	282	5.35	43.8	155	207	2.2	1.6			
Min	74.7	1.34534	227	3.22	26.0	22	87	1.4	1			
Max	84.7	1.34712	314	7.21	56.3	1,207	344	4.0	3			

[a] For each entry, the correlation coefficient (*r*) is in the top row, and the corresponding significance value is in the second row; *** indicates significance at $P \leq 0.0001$. Mean, minimum, and maximum values for each parameter from the fine-grind mill HWE are to the right and for the Congress mash are at the bottom. Correlations for the same analysis method are shown in bold.
[b] RI: refractive index; OC: osmolyte concentration; S/T: soluble/total; and FAN: free amino nitrogen.

TABLE III
Comparison of Correlations of Wort Parameters for 25-g Hot Water Extract (HWE) and 25-g Congress Mash for 28 Entries from the Western Regional Spring Barley Nursery from Crop Year 2008[a]

	Congress Mash[b]											
Fine-Grind Mill HWE[b]	**Malt Extract**	**RI**	**OC**	**Wort Protein**	**S/T Protein**	**β-Glucan**	**FAN**	**Color**	**Clarity**	**Mean**	**Min**	**Max**
Malt extract	**0.986**	0.976	0.699	0.573	0.870	–0.200	0.666	–0.040	–0.343	77.9	71.6	81.6
	***	***	***	$P = 0.001$	***	$P = 0.308$	***	$P = 0.840$	$P = 0.074$			
RI	0.981	**0.987**	0.748	0.642	0.883	–0.225	0.717	–0.036	–0.373	1.34591	1.34482	1.34651
	***	***	***	***	***	$P = 0.250$	***	$P = 0.856$	$P = 0.051$			
OC	0.891	0.918	**0.867**	0.726	0.877	–0.314	0.734	–0.142	–0.504	255	210	286
	***	***	***	***	***	$P = 0.104$	***	$P = 0.471$	$P = 0.006$			
Wort protein	0.540	0.609	0.761	**0.987**	0.653	–0.291	0.934	–0.115	–0.544	4.51	3.14	5.70
	$P = 0.003$	$P = 0.001$	***	***	$P = 0.0002$	$P = 0.133$	***	$P = 0.560$	$P = 0.003$			
S/T protein	0.886	0.903	0.769	0.722	**0.994**	–0.341	0.759	0.004	–0.458	36.7	23.9	50.5
	***	***	***	***	***	$P = 0.076$	***	$P = 0.984$	$P = 0.014$			
β-Glucan	–0.325	–0.338	–0.309	–0.337	–0.281	**0.945**	–0.265	0.280	0.440	219	51	889
	$P = 0.092$	$P = 0.078$	$P = 0.110$	$P = 0.080$	$P = 0.148$	***	$P = 0.173$	$P = 0.149$	$P = 0.019$			
FAN	0.708	0.752	0.725	0.913	0.766	–0.226	**0.960**	–0.101	–0.479	147	81	207
	***	***	***	***	***	$P = 0.248$	***	$P = 0.609$	$P = 0.010$			
Color	0.275	0.289	0.279	0.242	0.449	0.124	0.304	**0.491**	0.220	1.8	1.2	2.7
	$P = 0.157$	$P = 0.136$	$P = 0.151$	$P = 0.215$	$P = 0.017$	$P = 0.530$	$P = 0.116$	$P = 0.008$	$P = 0.261$			
Clarity	–0.335	–0.370	–0.461	–0.358	–0.283	0.283	–0.367	0.472	**0.595**	1.5	1.0	3.0
	$P = 0.081$	$P = 0.053$	$P = 0.014$	$P = 0.061$	$P = 0.145$	$P = 0.145$	$P = 0.055$	$P = 0.011$	$P = 0.001$			
Mean	79.9	1.34626	275	4.89	39.9	175	180	2.4	1.9			
Min	74.7	1.34534	228	3.44	26.0	31	93	1.4	1.0			
Max	83.4	1.34698	313	6.21	56.3	407	251	4.0	3.0			

[a] For each entry, the correlation coefficient (*r*) is in the top row, and the corresponding significance value is in the second row; *** indicates significance at $P \leq 0.0001$. Mean, minimum, and maximum values for each parameter from the fine-grind mill HWE are to the right and for the Congress mash are at the bottom. Correlations for the same analysis method are shown in bold.
[b] RI: refractive index; OC: osmolyte concentration; S/T: soluble/total; and FAN: free amino nitrogen.

Sample Analysis

Barley, CM, and standard-scale HWE were analyzed for common malting quality attributes using standard methodology (www.ars.usda.gov/SP2UserFiles/Place/36551000/barleyreports/2008MVN.pdf). In addition to standard malting quality measurements, CM and HWE worts were measured for refractive index (RI) using a digital refractometer (Mettler Toledo RE50) and osmolyte concentrations (OC) using a vapor pressure osmometer (Wescor model 5100c or 5520) following manufacturer instructions.

For small-scale HWE, soluble protein was measured as in Schmitt et al (21), and wort β-glucan concentrations were determined as in Schmitt and Budde (20). Extract (density) determinations for small-volume worts were made using manual sample introduction into a densitometer (Anton Paar DMA-4500M). Monitoring the sample compartment video display, combined with careful cell washing and drying between samples, allowed density measurements on samples of ≤1.0 mL, with sample recovery after measurement. Samples were also measured for RI and OC as above.

Free amino nitrogen (FAN) was determined in the small-scale extractions using a modification of the Skalar segmented flow analysis protocol routinely used for full-scale CM adapted to a 96-well microplate format. Briefly, wort samples and glycine standards (0–300 ppm) were diluted (11.5 μL of sample or standard + 188.5 μL of 0.3% Brij-35 solution) and mixed well in a V-bottom 96-well microplate. Diluted solutions (10 μL) were added to 60 μL of a 1.7 m*M* ascorbic acid solution in a semiskirted 96-well PCR plate, followed by addition of 80 μL of a 112 m*M* ninhydrin solution in 975 m*M* sodium acetate adjusted to pH 6.7 with acetic acid. The PCR plate was sealed using adhesive film (ThermalSeal), mixed on a thermomixer (Eppendorf Thermomixer-R), and transferred to a thermocycler (Eppendorf Mastercycler) preheated to 90°C. The plate was held at 90°C for exactly 15 min, rapidly cooled to 20°C using the maximum rate of thermocycler cooling, and held at 20°C for 5 min. Subsequently, 23 μL of a 9.3 m*M* KIO_3 solution was added to each well and mixed. A sample (100 μL) from each well was transferred to a clear, flat-bottom 96-well microplate. The absorbance of samples and standards was determined at 570 nm in a microplate reader (Molecular Devices Spectramax 340PC^{384}). FAN equivalents in the worts were calculated from the resulting standard curve.

Results from the two mashing methods (CM and HWE) were compared using a modification of our standard quality score rating system for CM worts (Table I). The modified quality scoring system for HWE used scoring windows that were adjusted to reflect overall differences in parameter values between CM and HWE. To compare the two mashing systems, the quality scores were compared using only wort-derived data (extract, wort soluble protein, S/T [soluble/total] protein, β-glucan, and wort clarity), not data (kernel weight, assortment, barley protein, DP, and α-amylase) normally used in the quality score generation but not derived from wort.

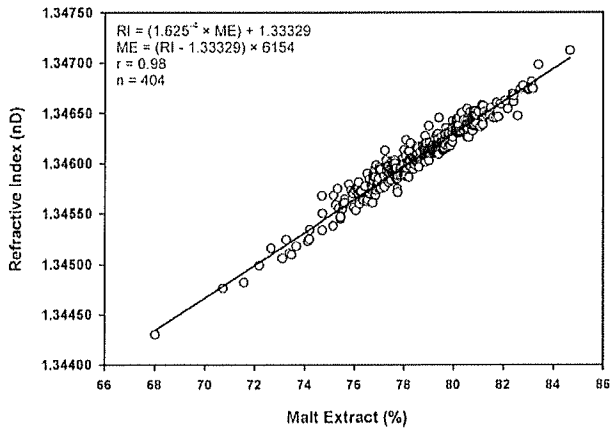

Fig. 1. Malt extract correlation with wort refractive index (RI). Comparisons were made between measured RI and malt extract calculations from combined data from the Mississippi Valley Regional and Western Regional Spring Barley nurseries from crop year 2008 derived from Congress mash, standard-scale hot water extract (HWE), and small-scale HWE worts. The equation for the calculated regression line was rearranged to show the estimation of malt extract from measured RI.

RESULTS

The 2008 crop year from MVN contained barley with diverse malting quality attributes (full nursery report available at www.ars.usda.gov/SP2UserFiles/Place/36551000/barleyreports/2008MVN.pdf). The MVN (108 entries) contained several malting barley varieties (6-row: Morex, Stander, Lacey, Tradition, and Stellar-ND; and 2-row: Harrington, Pinnacle, and Conrad), two feed varieties (Steptoe and Baronesse), and a number of experimental lines, all grown at four Midwestern locations (Fargo, Carrington, and Bottineau, ND, and Crookston, MN) as well as Aberdeen, ID. Barley samples averaged 12.7% protein (ranging from 9.5 to 16.3%). Across the lines tested, CM yielded malt extract values averaging 80.1% (ranging from 74.7 to 84.7%). A subset of 28 barley variety entries from WRSBN was evaluated as well and included Morex, Robust, Stander, Legacy, Harrington, Conlon, Conrad, Steptoe, and Baronesse, as well as experimental lines. CM worts from the selected lines from WRSBN had an extract range of 74.7 to 83.4% (average 79.9%).

To compare the two mashing protocols, we analyzed the worts produced by mashing the 108 entries from MVN and 28 selected lines from WRSBN using the two protocols and a set of malting quality parameters. Malting quality parameters included standard measures such as malt extract, wort soluble protein, wort S/T malt protein, wort β-glucan, FAN, wort color, and wort clarity, as well as nonstandard measures, including the RI and OC of the worts. Correlations among the results from MVN are shown in Table II, and results from WRSBN are shown in Table III. Also shown in Tables II and III are the means and ranges for each of the parameters for worts from the two mashing protocols. Across the range of measures and in both nursery experiments, HWE showed lower

TABLE IV
Comparison of Extract Values (Congress Mash) Using Grist from Three Grinding Methods[a]

	Grinding Method		
Line	FGM	BG	BB
5121	77.7	77.8	78.1
5124	80.5	79.8	80.5
5127	80.8	77.9	79.6
5128	80.1	77.7	79.2
5129	81.4	76.7	80.7
5131	82.2	78.7	82.8
5139	80.4	76.8	77.4
5144	80.8	77.3	81.2
5152	80.2	81.3	81.2
5154	83.2	81.7	84.3
5155	83.0	79.2	82.4
5157	80.1	78.4	80.0
5160	80.1	78.0	80.8
5162	80.0	78.5	79.7
Mean	80.8	78.6	80.6
SD	1.4	1.5	1.8
CV (%)	1.7	1.9	2.3

[a] FGM: standard ASBC Malt-4 fine-grind grist (1); BG: household coffee burr grinder adjusted to ASBC Malt-4 specifications; and BB: microscale ball mill (Mini Beadbeater-96).

solubilization than CM with lower extract, soluble protein, S/T protein, other solubles (RI and OC), and FAN, as well as higher β-glucan. However, despite the lower absolute values for the QA measures in HWE compared with CM, the individual attributes measured in the two worts for each line correlated extremely well across the majority of measured properties. Correlation coefficients generally were between 0.84 and 0.99 (MVN) and 0.87and 0.99 (WRSBN), except for wort color and clarity, which showed lower correlation coefficients (approx. 0.5–0.6) in both sample sets. Using the wort-derived parameters in Table I to generate quality scores for the two mashing methods for MVN, a comparable quality score correlation coefficient ($r = 0.89$) across the nursery lines and similar mean quality scores (19.0 [CM] versus 19.1 [HWE] out of a maximum possible score of 31.0) were obtained (data not shown).

In addition to the same-measure correlations, a number of other parameters correlated quite strongly with each other. Wort soluble protein and FAN showed tight correlations, as was expected (Tables II and III). Similarly, wort β-glucan showed negative correlations with other measures of modification and was only positively correlated with wort color and clarity (Tables II and III). CM viscosity was strongly correlated ($r > 0.9$) with β-glucan levels in both CM and HWE worts but negatively correlated with other measures of carbohydrate or protein modification (data not shown).

In particular, malt extract (based on wort density) and wort RI showed very strong correlations. Indeed, comparing paired malt extract values and RI measurements in both nurseries and across all three mashing protocols showed that the two measurements were extremely tightly linked (Fig. 1). This close, empirically determined relationship can also be rearranged to allow estimation of values of malt extract from measured RI values in the absence of direct specific gravity measurements, thus allowing estimates of malt extract using as little as 50 μL of wort.

We were also interested in evaluating the potential for small-scale HWE to predict performance in both standard-scale HWE and standard-scale CM. In previous work (21), we successfully miniaturized the CM protocol, using approx. 1% of the grist and volume of our standard-scale CM, while still producing results that were comparable to the standard-scale mashing system. To streamline small-

TABLE V
Comparison of Wort Analysis Results for Small-Scale (0.188 g) Hot Water Extract (HWE) (Samples Ground with a Microscale Ball Mill) and Standard-Scale (25 g) HWE and Congress Mash (Samples Ground with Standard Fine-Grind Mill) for Lines from the Mississippi Valley Regional Barley Nursery[a]

Microscale Ball Mill HWE[b]	Fine-Grind Mill HWE[b]									
	Malt Extract	RI	OC	Wort Protein	S/T Protein	β-Glucan	FAN	Mean	Min	Max
Malt extract	**0.852**	0.812	0.551	0.149	0.579	−0.133	0.228	77.5	68.0	82.6
	***	***	***	$P = 0.125$	***	$P = 0.173$	$P = 0.018$			
RI	0.812	**0.852**	0.634	0.271	0.674	−0.211	0.376	1.34589	1.34430	1.34647
	***	***	***	$P = 0.005$	***	$P = 0.029$	***			
OC	0.421	0.533	**0.715**	0.616	0.688	−0.317	0.670	251	149	292
	***	***	***	***	***	***	***			
Wort protein	0.235	0.359	0.746	**0.910**	0.727	−0.170	0.857	4.61	2.59	6.25
	$P = 0.014$	***	***	***	***	$P = 0.080$	***			
S/T protein	0.623	0.700	0.805	0.586	**0.922**	−0.175	0.679	37.7	21.7	50.9
	***	***	***	***	***	***	***			
β-Glucan	−0.198	−0.304	−0.399	−0.339	−0.350	**0.766**	−0.394	173	24	1187
	$P = 0.040$	$P = 0.001$	***	$P = 0.0004$	$P = 0.0002$	***	***			
FAN	0.175	0.285	0.677	0.816	0.632	−0.017	**0.763**	208	79	327
	$P = 0.072$	$P = 0.003$	***	***	***	$P = 0.861$	***			
Mean	78.1	1.34598	262	4.92	40.2	209	170			
Min	71.6	1.34482	210	2.86	23.9	46	81			
Max	82.3	1.34666	291	6.54	50.5	1,350	258			

Microscale Ball Mill HWE[b]	Fine-Grind Mill Congress Mash[b]						
	Malt Extract	RI	OC	Wort Protein	S/T Protein	β-Glucan	FAN
Malt extract	**0.809**	0.715	0.412	0.156	0.575	0.117	0.268
	***	***	***	$P = 0.112$	***	$P = 0.233$	$P = 0.006$
RI	0.764	**0.729**	0.489	0.284	0.673	0.006	0.357
	***	***	***	$P = 0.003$	***	$P = 0.954$	$P = 0.0002$
OC	0.397	0.508	**0.590**	0.636	0.688	−0.372	0.623
	***	***	***	***	***	***	***
Wort protein	0.226	0.415	0.726	**0.903**	0.693	−0.204	0.867
	$P = 0.021$	***	***	***	***	$P = 0.034$	***
S/T protein	0.626	0.611	0.663	0.650	**0.832**	−0.643	0.656
	***	***	***	***	***	***	***
β-Glucan	−0.121	−0.185	−0.271	−0.321	−0.321	**0.697**	−0.259
	$P = 0.220$	$P = 0.059$	$P = 0.005$	$P = 0.001$	$P = 0.001$	***	$P = 0.008$
FAN	0.192	0.344	0.669	0.820	0.612	−0.124	**0.783**
	$P = 0.050$	$P = 0.0003$	***	***	***	$P = 0.124$	***
Mean	80.1	1.34633	282	5.35	43.8	155	207
Min	74.7	1.34534	227	3.22	26.0	22	87
Max	84.7	1.34712	314	7.21	56.3	1,207	344

[a] For each entry, the correlation coefficient (r) is in the top row, and the corresponding significance value is in the second row; *** indicates significance at $P \leq 0.0001$. Mean, minimum, and maximum values for each parameter from the microscale ball mill HWE are to the right and for the fine-grind mill HWE and Congress mash are at the bottom of each section. Correlations for the same analysis method are shown in bold.

[b] RI: refractive index; OC: osmolyte concentration; S/T: soluble/total; and FAN: free amino nitrogen.

scale mashing systems, we examined two alternate approaches to sample grinding (a procedural bottleneck) that are potentially better matched to small sample sizes. In the first approach, we used an adjustable burr mill-type household coffee grinder to generate grist with the same size characteristics (10 ± 1% retention on a no. 30 sieve after 3 min on a mechanical shaker) as the standard laboratory mill. The grist produced consisted of a variety of grain particles, including relatively large hull fragments, similar to the grist from the standard laboratory mill, with nominally similar particle size distribution to the standard laboratory mill (data not shown). The coffee mill was well suited to smaller sample quantities compared with the standard-scale mill. The second approach used a small-scale ball mill (Mini Beadbeater-96). In this system, malt samples and stainless-steel balls are contained in 2.0-mL microcentrifuge tubes. Sample containers are vigorously shaken back and forth, and the sample is crushed by the kinetic energy of the balls as they impact the sample. Malt samples can be quite finely ground, with only small fragments of the hull remaining. Sample retention characteristics were not determined for the microscale ball milled samples, although the ground malt was visually finer in appearance than ground material from the other sample mills. CM worts from a series of malts ground using the three methods were prepared, and values for malt extract were determined. Table IV shows that, despite milling the grist to the same specifications, the household coffee burr grinder resulted in CM extract values approx. 2% lower on average than the standard fine-grind grist. The reasons for this discrepancy are unclear. In contrast, the worts from grist ground using the small-scale ball mills resulted in mean extract values similar to the standard grist (80.8 versus 80.6%), with comparable sample CV. As a result, samples for small-scale HWE wort preparations used the finely ground grist from the microscale ball mills.

Small-scale HWE worts were prepared, and the results of wort analysis were compared with those from the standard-scale HWE and CM from the two regional nurseries (Tables V and VI). In most cases, mean values for the wort parameters generated using the three methods differed, with values from the CM > full-scale HWE > small-scale HWE. Correlations between the malting quality attributes from the small-scale and full-scale HWE for MVN were quite

TABLE VI
Comparison of Wort Analysis Results for Small-Scale (0.188 g) Hot Water Extract (HWE) (Samples Ground with a Microscale Ball Mill) and Standard-Scale (25 g) HWE and Congress Mash (Samples Ground with Standard Fine-Grind Mill) for Lines from the Western Regional Spring Barley Nursery[a]

Microscale Ball Mill HWE[b]	Fine-Grind Mill HWE[b] Malt Extract	RI	OC	Wort Protein	S/T Protein	β-Glucan	FAN	Mean	Min	Max
Malt extract	**0.950** ***	0.933 ***	0.851 ***	0.519 $P = 0.005$	0.816 ***	–0.433 $P = 0.021$	0.663 ***	77.2	70.7	80.8
RI	0.957 ***	**0.952** ***	0.873 ***	0.603 $P = 0.001$	0.870 ***	–0.402 $P = 0.034$	0.753 ***	1.34579	1.34476	1.34637
OC	0.518 $P = 0.005$	0.553 $P = 0.002$	**0.612** $P = 0.001$	0.581 $P = 0.001$	0.526 $P = 0.004$	–0.342 $P = 0.075$	0.581 $P = 0.001$	236	149	273
Wort protein	0.655 $P = 0.0002$	0.713 ***	0.792 ***	**0.896** ***	0.792 ***	–0.312 $P = 0.106$	0.902 ***	4.12	2.95	5.42
S/T protein	0.851 ***	0.854 ***	0.847 ***	0.538 $P = 0.003$	**0.959** ***	–0.254 $P = 0.192$	0.691 ***	40.2	23.9	50.5
β-Glucan	–0.532 $P = 0.004$	–0.557 $P = 0.002$	–0.552 $P = 0.002$	–0.514 $P = 0.005$	–0.466 $P = 0.012$	**0.901** ***	–0.477 $P = 0.010$	234	36	1,187
FAN	0.579 $P = 0.001$	0.636 $P = 0.0003$	0.723 ***	0.748 ***	0.726 ***	–0.065 $P = 0.724$	**0.810** ***	172	118	272
Mean	77.9	1.34591	255	4.51	36.7	219	147			
Min	71.6	1.34482	210	3.14	23.9	51	81			
Max	81.6	1.34651	286	5.70	50.5	889	207			

Microscale Ball Mill HWE[b]	Fine-Grind Mill Congress Mash[b] Malt Extract	RI	OC	Wort Protein	S/T Protein	β-Glucan	FAN
Malt extract	**0.931** ***	0.910 ***	0.634 $P = 0.0003$	0.550 $P = 0.002$	0.808 ***	–0.269 $P = 0.166$	0.629 $P = 0.0003$
RI	0.947 ***	**0.939** ***	0.688 ***	0.636 $P = 0.0003$	0.862 ***	–0.234 $P = 0.231$	0.711 ***
OC	0.566 $P = 0.002$	0.585 $P = 0.001$	**0.483** $P = 0.009$	0.615 $P = 0.001$	0.539 $P = 0.003$	–0.431 $P = 0.022$	0.573 $P = 0.001$
Wort protein	0.684 ***	0.735 ***	0.764 ***	**0.927** ***	0.791 ***	–0.235 $P = 0.229$	0.891 ***
S/T protein	0.877 ***	0.880 ***	0.684 ***	0.604 $P = 0.001$	**0.971** ***	–0.289 $P = 0.136$	0.644 $P = 0.0002$
β-Glucan	–0.475 $P = 0.011$	–0.489 $P = 0.008$	–0.427 $P = 0.023$	–0.927 ***	–0.435 $P = 0.021$	**0.731** ***	–0.437 $P = 0.020$
FAN	0.630 $P = 0.0003$	0.695 ***	0.675 ***	0.787 ***	0.732 ***	–0.124 $P = 0.530$	**0.779** ***
Mean	79.9	1.34626	275	4.89	39.9	175	180
Min	74.7	1.34534	228	3.44	26.0	31	93
Max	83.4	1.34698	313	6.21	56.3	407	251

[a] For each entry, the correlation coefficient (r) is in the top row, and the corresponding significance value is in the second row; *** indicates significance at $P \leq 0.0001$. Mean, minimum, and maximum values for each parameter from the microscale ball mill HWE are to the right and for the fine-grind mill HWE and Congress mash are at the bottom of each section. Correlations for the same analysis method are shown in bold.

[b] RI: refractive index; OC: osmolyte concentration; S/T: soluble/total; and FAN: free amino nitrogen.

good. All of the same-measure correlations were very highly significant ($P \leq 0.0001$), with correlation coefficients ranging from a low of 0.71 for OC to >0.9 for wort protein and S/T protein (Table V). Comparisons of individual parameters for lines from WRSBN (Table VI) were similarly very highly significant, with correlation coefficients higher for malt extract and RI, lower for OC, and marginally higher for the remaining comparisons (wort protein, S/T protein, β-glucan, and FAN).

Extending the comparisons further, individual parameter results from small-scale HWE correlated well with standard-scale (25 g) CM worts for both MVN and WRSBN (Tables V and VI). With a single exception, all comparisons between small-scale HWE and standard-scale CM were still very highly significant ($P \leq 0.001$), with correlation coefficients ranging from 0.7 to 0.9. In both nurseries, comparisons between OC in small-scale HWE and standard-scale CM were the weakest, with correlation coefficients of 0.6 and 0.5 in MVN and WRSBN, respectively. Nonetheless, even the lowest correlations of OC measurements were significant at $P \leq 0.0001$ and 0.01 for MVN and WRSBN, respectively. Using the primary malting quality measures for wort (malt extract, wort soluble protein, S/T protein, and wort β-glucan) and the ratings scales above, correlations between quality scores calculated from the small-scale HWE and full-scale CM data resulted in correlation coefficients of 0.64 for the lines from MVN and 0.75 for the lines from WRSBN (data not shown).

Given the strong correlations between full-scale HWE and CM, we examined whether it would be possible to use HWE results to predict the CM value. Figure 2 shows the linear regressions for same-measure results for comparison of six wort parameters between standard-scale HWE and CM. The regression equation was rearranged to show CM values as a function of HWE measurements. Although the numeric values for the wort measurements from the two mashing protocols differed somewhat, the tight correlations between the two methods for malt extract, soluble protein, β-glucan, and RI allowed reasonable prediction of the results for one mashing protocol based on the results from the other. Given these highly correlated measures, estimating the likely malt extract value for CM from the corresponding malt extract value for the simpler HWE should be feasible, with analogous relationships for wort parameters, including soluble protein, β-glucan, and RI. The linear regressions for FAN and OC between CM and HWE showed more variability than those for malt extract, soluble protein, β-glucan, and RI, although the correlations were still highly significant (Table II), with HWE measures still predictive of CM values.

Similar comparisons of results from small-scale HWE with those of standard-scale CM are shown in Figure 3. In general, the replicate mashing and analyses using small-scale HWE provided excellent reproducibility among replicates, with only a few samples showing substantial standard deviations for RI or FAN. The low within-sample standard deviations for replicate analyses for the majority of samples from small-scale HWE, with only a few samples showing substantial variation, might allow analyses to be conducted without replication, depending on the acceptable level of sample error. The lower correlations for the small-scale HWE and CM data sets in Table V and shown in Figure 3, compared with the standard-scale HWE and CM comparison (Table II; Fig. 3) suggest that the reduction in mashing sample size resulted in greater error in the calculated values. Nonetheless, results from the standard scale and those from small-scale analyses were still very well correlated at $P < 0.0001$ (Table V), with the small-scale HWE worts predictive of the relative performance of standard-scale CM.

DISCUSSION

Malting quality is governed by a complex set of traits, most of which involve a series of physiological or biochemical processes. Members of the brewing and malting industries have developed several series of standard analyses and procedures, the malting QA methods that underlie the production and commerce of barley malt by quantifying different aspects of malt performance. By extension, these malting QA methods also set the standards that new malting barley lines must meet (or exceed) to be accepted by growers, maltsters, and brewers. At the end of germplasm development, new lines must meet these established criteria and show acceptable performance at malting and brewing companies to join the ranks of accepted malting barley varieties. To pass this hurdle, new lines must pass rigorous testing against a range of malting performance standards to ensure that the lines will perform adequately and reliably.

Malting quality performance information is also necessary at the early stages of line development. However, in such early-stage germplasm testing, it is not essential to fully detail how any given line performs. Rather, initial malting assessments should help identify those lines that are likely to meet performance benchmarks, but also, importantly, identify those lines that show deficiencies in critical elements of malting performance. Simplified screening methods for a few key characteristics could have advantages in initial evaluations of breeding lines if the screening methods allow better use of malting QA resources or decrease data turnaround time, while still providing useful data for culling unproductive crosses. Simplified QA methodology that is still predictive of performance in standard tests would also benefit laboratories that do not have ready access to dedicated malting quality assessment infrastructure by enabling limited malting quality assessments without the major expenses involved in setting up and operating a full-scale QA laboratory.

Worts from a HWE method generally yield different values for standard QA metrics than do worts from a CM method, with HWE producing lower estimates for malt extract, soluble protein, and S/T protein, as well as higher wort β-glucan (Table III). As a result, the IoB HWE mashing cycle (18) is infrequently used, as the results of analyses of these worts are not immediately comparable to standards set using a CM method. However, despite the differences in the values for the QA metrics for the two mashing protocols, there are excellent correlations between a number of the metrics (Table III; Fig. 2), with HWE results highly predictive of CM results. Summary quality scores estimated for the two types of worts produced from 108 lines from MVN from crop year 2008 show a strong correlation coefficient, requiring only minor adjustments in the ratings scale from that normally used for scoring CM worts. This suggests that the simpler HWE could serve as a useful proxy for standard CM when identifying malts with good malting quality potential.

The HWE methods examined here have a marginal efficiency advantage over the CM method for fully equipped and staffed QA labs in that the HWE mash protocol runs in half the time of the CM protocol and can be similarly analyzed on routine QA instrumentation. This could decrease sample analysis cycle times somewhat. However, the most significant advantage to using HWE would be for those laboratories lacking the mash bath control systems needed to execute the CM temperature profile. The simple instrumentation needed to hold a fixed temperature and mix container contents to conduct the HWE mashing protocol is less expensive and more widely available than the programmable heating, cooling, and stirring baths needed for CM, making malting quality analyses feasible outside dedicated malt QA laboratories.

The scale of samples and corresponding instrumentation used for such an isothermal mashing protocol can vary widely from the 25-g standard mash systems used here in the full-scale HWE, to comparable 0.5–1.0 g (5–10 mL) vortexed samples used by Fox and Henry (14), to simple 3-mL stirred isothermal dry-block incubations (data not shown), to the 1.5-mL scale used in this study, and likely other configurations as well. Although there are advantages to the small-scale (1.5 mL) mashing protocols (small sample re-

quirements, adaptability to standard laboratory instrumentation, and sample handling efficiencies related to the 96-well microplate format), there are also advantages to using the standard 25-g sample size. The primary advantage of the full-scale (25-g) procedures over reduced-scale mashing protocols is that larger quantities of malt can reduce sample variability, dampening any grain-to-grain variations in malting quality characteristics by sampling larger numbers of malt grains. In contrast, the microscale ball mill grinding method used here to overcome a significant sample analysis bottleneck has the disadvantage of a limited sample size. Sampling the 200 mg of malt in an individual tube for the microscale ball mill grinding method translates to choosing 4–5 individual malt grains for homogenization and analysis compared with the 400–500 individual malt grains analyzed in a 25-g sample. As a result, sample heterogeneity and sampling error could have a significantly greater effect when working at the very small scales used here compared with

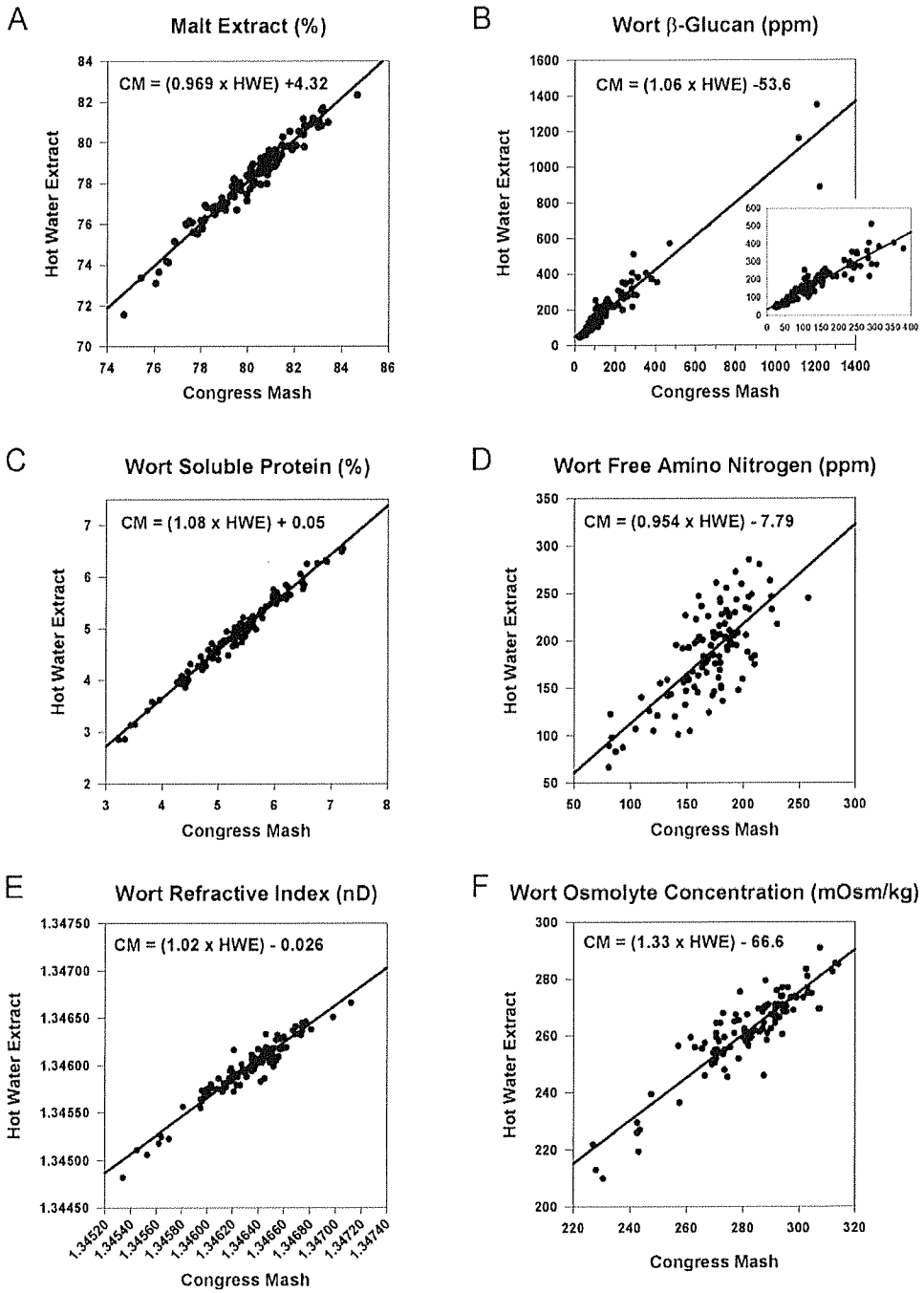

Fig. 2. Linear regressions calculated from Congress mash (CM) and standard-scale hot water extract (HWE) worts from Mississippi Valley Regional Nursery lines for malt extract and wort β-glucan, soluble protein, free amino nitrogen, refractive index, and osmolyte concentration (**A–F**). Regression coefficients were rearranged to allow estimation of CM wort values from HWE wort results. Results for wort β-glucan are shown over the entire range measured (**B,** main view), as well as the more commonly encountered 0–400 ppm range (**B,** inset). Mashing and sample analyses for CM and standard-scale HWE were not replicated.

the bulk samples in a standard-scale mash. Despite the significant sample-to-sample variation seen in a few instances in Figure 3D and E, however, standard deviations for most of the data points used in the various panels in Figure 3 were quite low, suggesting that the potential negative effects of small sample sizes are neither universal nor prohibitive. Scaling down HWE worts by approx. 99% to a 2.0-mL microcentrifuge tube system and analysis of wort parameters at this small scale on standard laboratory instrumentation allows remarkably good predictions of how various malts would perform in standard-scale CM (Tables V and VI; Fig. 3), while avoiding the need for dedicated mash baths, filtration stations, and automated wort-analysis systems. Although correlations between CM and small-scale HWE QA results were not as high as the correlations for full-scale CM and HWE, they were still highly signifi-

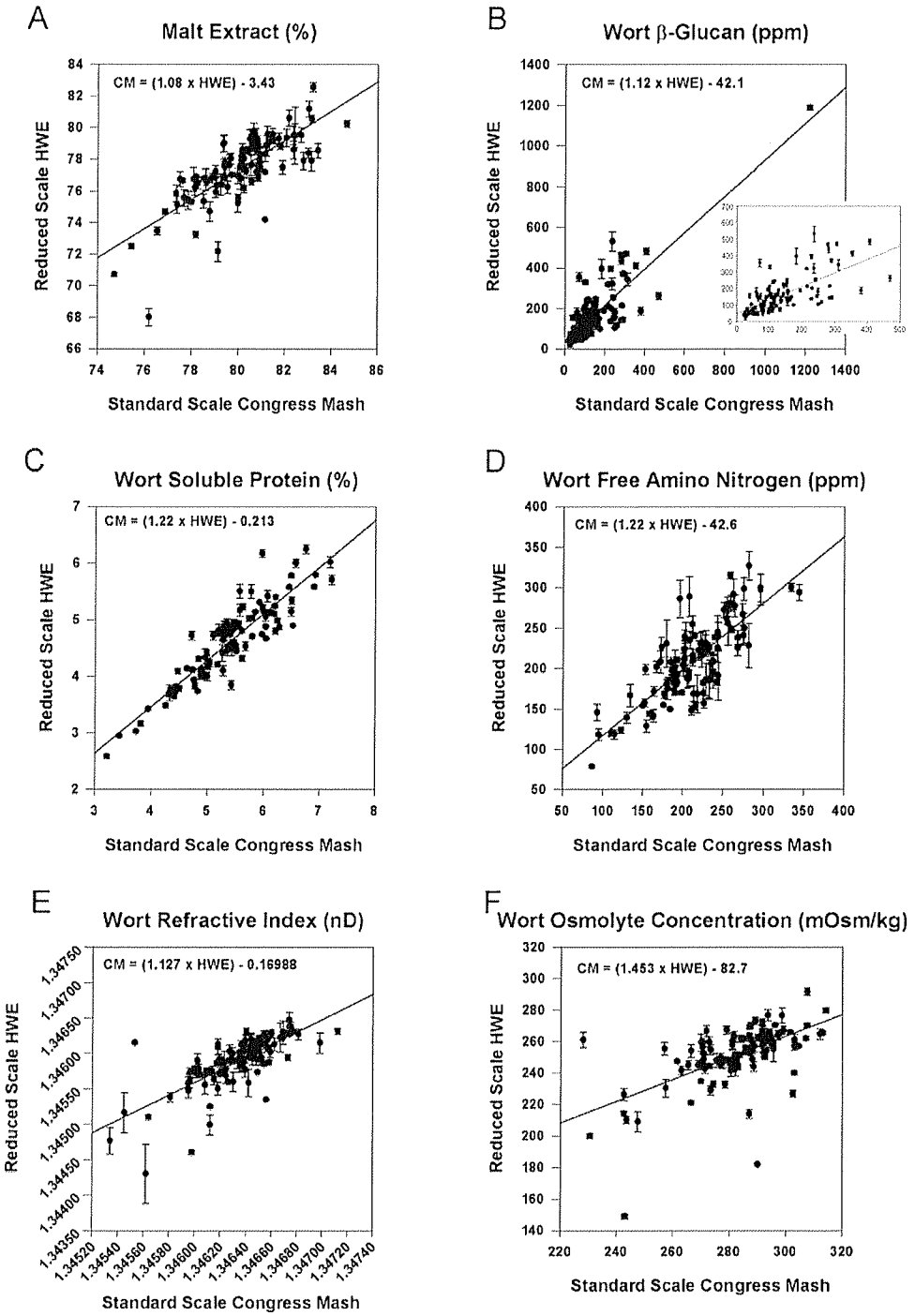

Fig. 3. Linear regressions calculated from paired Congress mash (CM) and reduced-scale hot water extract (HWE) worts from Mississippi Valley Regional Nursery lines for malt extract and wort β-glucan, soluble protein, free amino nitrogen, refractive index, and osmolyte concentration (**A–F**), including wort β-glucan plots over the full range of data (**B,** main view) and commonly encountered concentrations (**B,** inset). Regression coefficients were rearranged to allow estimation of CM wort values from HWE wort results. Error bars for reduced-scale HWE values indicate standard deviation for three replicates. Where not visible, error bars are smaller than the symbols used. Standard-scale CM data shown were not replicated.

cant and easily identified the best and worst of the malts tested, meeting the needs of an initial QA screen.

The two additional parameters measured (RI and OC) also offer advantages. RI, which has been used in the past to measure wort extract (8), is readily and easily measured with high accuracy and precision using digital refractometers. References to historical literature spanning many decades (14) suggest that the two measurements (density and RI) have waxed and waned in popularity for malt extract measurements. Today, both types of instruments are available and provide high precision, accuracy, and ease of use. High-quality digital refractometers can be significantly less expensive than the digital density meters commonly used for extract determination in malt QA laboratories. The very high correlations between density (malt extract) and RI measurements in standard-scale mashes (Tables II and III) suggest that use of such digital refractometers may provide a valid and less expensive, practical alternative to traditional density measurements for extract determinations. Indeed, the data presented in Figure 1 show that the two measurements of density and RI seem to be interchangeable, with values readily converted from one measurement to the other. For worts of sufficient volume, autosamplers are available for each class of instrument. For small-scale worts (both HWE presented here and CM presented previously [21]) with sample sizes of approx. 1 mL, standard autosamplers are not feasible. However, new density meters from several vendors use video cameras to view the measurement chamber, facilitating manual sample loading. The cameras enable use of these meters in a manual sample-loading mode with approx. 1 mL of wort. However, manual sample loading, recovery, and cell cleaning becomes tedious for multiple samples. For such limited-quantity worts, use of automatic digital refractometers, with sample requirements of a few tens of microliters, offers a rapid and convenient alternative to density meter extract determination.

OC, which is also readily quantified for small volumes using relatively inexpensive instrumentation, is a measure of the concentration of osmotically active molecules such as sugars (dominating in malts and worts) and amino acids. Data in this report show that OC in both HWE and CM worts from several regional nurseries is closely correlated to malt extract, wort soluble protein, and other critical malting quality measurements and, thus, may become a valuable malting quality metric in the future. The correlations of CM and HWE wort OC with other malting quality parameters tended to be somewhat lower than other traditional metrics, as well as earlier evaluations of OC as a malting quality indicator (6,15). This may suggest that neither CM nor HWE may be optimal for linking OC with other malting quality parameters. Also, it is worth noting that Henson and Duke (15) found the lowest correlation between OC, malt extract, and S/T protein at day 5 of germination, the point at which the malts in this study were kilned. As a result, data presented here may have underestimated the utility of OC measurements for estimating malting quality.

Implementing small-scale HWE and associated analyses may also provide a nimble option for relatively quick measurement of a few key malting quality parameters. The combination of a mixing and heating block, microcentrifuge, and digital refractometer could provide a researcher with a simple means of measuring malt extract values, one of the most important malting quality attributes, simply and relatively inexpensively using limited amounts of malt. Addition of microplate format fluorometers, spectrophotometers, or osmometers as resources allow would facilitate expansion of the analyses to include other malting quality parameters. Such simple assessments could be particularly important for regional barley improvement programs in areas without ready access to malting QA infrastructure. Additionally, a simple procedure using a single malt quality parameter, or one expanded to include a few additional traditional QA parameters, such as α-amylase and diastatic power, or novel measurements, such as OC or diastatic power enzymes, could be adapted to a classroom or teaching laboratory to provide an introduction to the basics of malt quality analysis in a hands-on setting. The small sample requirements of small-scale mashing and analysis procedures could also be useful in those instances where malt QA data are desired but sample availability is substantially limited, as with special genetic populations.

Barley breeders and members of the malting and brewing industries are both customers of malting quality analyses. However, looking carefully at the needs of malting quality analysis customers at the two ends of the malting barley development pipeline shows that their needs are not identical. Barley breeders screen hundreds or thousands of progeny seeking to identify those few individual lines that possess a desirable malting quality (and agronomic and disease resistance) profile, while maltsters and brewers need to evaluate and understand in detail the properties of a few lines that have survived to the end of the long selection process. These different uses of malting quality data may suggest situations in which modifications of standard malt QA protocols may be useful.

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